PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/17, 39/145

A1

(11) International Publication Number: WO 95/31996

(43) International Publication Date: 30 November 1995 (30.11.95)

(21) International Application Number: PCT/US95/06689

(22) International Filing Date: 25 May 1995 (25.05.95)

(30) Priority Data:
08/249,175 25 May 1994 (25.05.94) US

(71) Applicant: MILKHAUS LABORATORY [US/US]; Corner of Westfall & Larry Hill Road, R.D. 1, P.O. Box 127, Delanson, NY 12053 (US).

(72) Inventors: KLINE, Ellis, L.; 203 N. Elm, Pendleton, SC 29670 (US). McMICHAEL, John; Corner of Westfall & Larry Hill Road, R.D. 1, P.O. Box 127, Delanson, NY 12053 (US).

(74) Agent: SHARP, Jeffrey, S.; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 S. Wacker Drive, Chicago, IL 60606-6402 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

66

(54) Title: MATERIALS AND METHODS FOR TREATMENT OF PLAQUING DISEASES

(57) Abstract

Methods and compositions are provided for alleviation of disease states involving plaque formation, such as are manifested in Alzheimer's Disease and other amyloid disorders, and arteriosclerotic disease.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

MATERIALS AND METHODS FOR TREATMENT OF PLAQUING DISEASES

FIELD OF THE INVENTION

The present invention relates to methods and materials for the treatment of diseases involving plaque formation.

BACKGROUND OF THE INVENTION

Cellular skeletal systems have three distinct ultrastructural features, microtubules, intermediate filaments, and microfilaments, all of which are fibrous macromolecules associated with the central nervous system (CNS). Neuronal intermediate filaments, defined as neurofilaments (containing amyloid beta protein constructs), are distinct from other intermediate filaments found in the cells of the central nervous system. R.D. Goldman, A. Milstead, J.A. Schloss and M.J. Yerna, *Annu. Rev. Physiol.*, 41, p. 703-722 (1979); R.J. Lasak, *Neurosci. Res. Program Bull.*, 19, p. 7-32 (1981); R.J. Lasek and M.L. Shelanski, *Neurosci. Res. Program Bull.*, 19, p. 3-153 (1981); C.A. Maretta, ed., *Neurofilaments* (1983); M.L. Shelanski and R.K.H. Liem, *J. Neurochem.*, 33, p. 5-13 (1979). Neurofilaments are composed of three proteins with molecular weights of 200,000, 150,000 and 70,000 daltons. B.H. Toh, L.J. Gibbs, Jr., D.C. Gajdusek, J. Goudsmit and D. Dahl, *Proc. Natl. Acad. Sci. USA*. An additional 62,000 dalton protein is also affiliated with the above-mentioned proteins. Such proteins are associated with slow axoplasmic

transport. P.N. Hoffman and R.J. Lasek, *J. Cell Biology*, 66, p. 351-366 (1975).

Alzheimer's Disease, and other amyloid associated maladies including senile dementia, Down's syndrome, Pick's disease, progressive supranuclear palsy, multiple sclerosis and others, are characterized by the presence of one or more fused fibrils of repetitive amyloid beta proteins or other similar amyloid residues such as paired helical filaments, neurofibrillary tangles, neuritic plaques, amyloid plaques and cerebrovascular amyloidosis. B.H. Anderson, D. Breinberg and M.J. Downes, *Nature*, 298, p. 84-86 (1982). These paired helical filaments are indistinguishable immunologically and chemically from normal neurofilaments and share many of the same proteinaceous epitopes. B.H. Anderson, D. Breinberg and M.J. Downes, *Nature*, 298, p. 84-86 (1982); B.H. Toh, L.J. Gibbs, D.C. Gajdusek, J. Goudsmit and D. Dahl, *Proc. Natl. Acad. Sci. USA*; K. Iqbal, I. Grundke-Iqbal, H.M. Wisnieski and R.D. Terry, *Brain Res.*, 142, p. 321-332 (1975). It has been suggested that they interfere with axonal transport. P.N. Hoffman and R.J. Lasek, *J. Cell. Biol.*, 66, p. 351-366 (1975); J.W. Griffin, P.N. Hoffman, A.W. Clark, P.T. Carroll and D.L. Price, *Science*, 202, p. 633-665 (1978).

Using a cDNA clone of the gene encoding amyloid beta protein as a genetic probe, it was shown that the gene is located on chromosome twenty-one and is expressed in many tissues of the body. D. Goldjaber, M.I. Lerman, O.W. McBridge, U. Suffiotti and D.C. Gaidusak, *Science*, 235, p. 77-780 (1987); R.E. Tanzi, J.F. Gusella, P.C. Watkins, G.A.P. Bruns, P. St.George, M.L. Vankeuren, D. Patterson, S. Pagan, D.M. Kurnit and R.L. Neve, *Science*, 235, p. 880-884 (1987). Quantitation of amyloid beta protein expression, as seen by its mRNA levels using the cDNA probe, has revealed that its level of expression in brain tissue of Alzheimer's patients was not above that seen for other tissues outside the central nervous system. Such a finding was of interest

to researchers when noting that amyloid plaque formation only occurs in the brain. R.E. Tanzi, J.F. Gusella, P.C. Watkins, G.A.P. Bruns, P. St.George, M.L. Vankeuren, D. Patterson, S. Pagan, D.M. Kurnit and R.L. Neve, *Science*, 235, p. 880-884 (1987).

Amyloid beta protein is obtained through conventional means known in the art and has been characterized in various reports. A.S. Cohen and E. Calkins, *Nature*, 183, p. 1202 (1959); A.S. Cohen and E. Calkins, *J. Cell Biology*, 21, p. 481 (1964); A.S. Cohen, E. Calkins, and C. Levens, *Am. J. Pathol.*, 35, p. 979 (1959). More recent work is manifested by D. Caspi, M.C. Baltz and M.K. Pepys, *Mol. Biol. Med.*, 3, pp. 387-407 (1986); and D. Caspi, M.C. Baltz and M.K. Pepys, *Mol. Biol. Med.*, 3, pp. 409-424 (1986). Amyloid beta protein exists in various structural forms. The amyloid beta protein that has been experimentally used and as referred to herein in terms of any specific embodiments constitutes a mixture of such forms. It is to be understood that within the scope of the present invention, it is contemplated that any of the various forms of amyloid beta protein may be used.

Amyloid beta protein from the brain has been cDNA cloned and shown to contain a unique twenty amino acid NH_2-terminal sequence. Glenner, G.G. and Wong, W., *Biochem. Biophys. Res. Comm.*, 122, No. 3, pp. 1131-35 (1984); D. Caspi, M.C. Baltz and M.K. Pepys, *Mol. Biol. Med.*, 3, pp. 409-424 (1986); Goldgaber, D., Lerman, M.I., McBridge, O.W., Saffiotti, U. and Gaidusak, D.C., *Science*, 235, pp. 777-80 (1987).

It has been observed that a buildup of abnormally organized amyloid beta protein in brain tissue is manifested in Alzheimer's Disease. See Dennis J. Selkoe and Carmela R. Abraham, "Isolation of Paired Helical Filaments and Amyloid Fibers from Human Brain," 134, *Methods in Immunology*, 388-404 (1986). The fact that there is an accumulation of beta amyloid protein in the brain in Alzheimer patients has been demonstrated by post mortem analysis of brain tissue that manifest a

concentration of amyloid beta protein as part of an accumulation of parallel filaments or neural fibrillatory tangles in the brain that appear characteristic of Alzheimer victims, along with neuritic plaque and cerebral vasculatory amyloidosis.

The presence of amyloid beta protein in fibrils and plaques in Alzheimer's Disease, as well as other CNS disorders, has been suggested to be a result of a degradation product of the normal neurofilaments, D. Goldjaber, M.I. Lerman, O.W. McBridge, U. Syffiotti and D.C. Gaidusak, *Science*, 235, p. 77-780 (1987); R.E. Tanzi, J.F. Gusella, P.C. Watkins, G.A.P. Bruns, P. St.George, M.L. Vankeuren, D. Patterson, S. Pagan, D.M. Kurnit and R.L. Neve, *Science*, 235, p. 880-884 (1987); M. Baudry, B.R. Dubrin, L. Beasley, M. Leon and G. Lynch, *Neurobiol. Aging*, 7, p. 255-260 (1986); G.G. Glenner, *Arch. Path. Lab. Med.*, 107, p. 218-282 (1983); or possibly due to improper metabolism of byproducts. Further breakdown products of amyloid beta proteins from neurofilaments have also been observed in amyloid plaques along meningeal vascular walls and intracortical blood vessels. S. Bahmanyar, E.J. Williams, F.B. Johnson, S. Young and D.C. Gaidusak, *J. Comp. Path*, 95, p. 1-5 (1985); M.E. Bruce and H. Fraser, *Neuropathol. Appl. Neurobiol*, 1, p. 189-207 (1981); M.E. Bruce and H. Fraser, *Neurophathol. Appl. Neurobiol.*, 7, p. 289-298 (1981); G.G. Glenner and W. Wong, J. Quaranta and G.G. Glenner, *Proc. Natl. Acad. Sci.*, 82, p. 8729 (1985); D.J. Selkoe, C.R. Abraham, M.B. Podlisky and L.K. Duffy, *J. Neurochem.*, 46, p. 1820 (1986).

During the mid-1960's, Solomon & Moos speculated that there was a close integration between immunological function, the central nervous system, psychophysiological factors (emotions), and disease, both physical and mental. G.F. Solomon and R.H. Moos, *Arch. Gen. Psychiatry*, 11, p. 657-674 (1964). The integration of those systems was initially suggested through observation of the presence of abnormal

immunoglobulins in schizophrenic patients. G.F. Solomon and R.H. Moos, *Arch. Gen. Psychiatry*, 11, p. 657-674 (1964); J.G. Knight, *Lancet*, 82, p. 1073-1076 (1982); W.J. Fessel and M. Hirata-Hibi, *Arch. Gen. Psychiatry*, 9, p. 601-613. These immune aberrations (termed autoantibodies), which seemed to target certain body cellular structures, G.F. Solomon, *Psychoneuroimmunology*, p. 259-278 (1985); G.F. Solomon and R.H. Moos, *Psychosom. Mod.*, 27, p. 135-149 (1981), supported the concept that there is a close communication between the CNS and the immune system. For instance, met-enkephalin is a neurotransmitter in the CNS and is a product of activated T-helper cells. G. Zurawaki, M. Benedik, D.J. Kamb, J.S. Abrams, S.M. Zurawaki and F.O. Lee, *Science*, 232, p. 772-775 (1986).

The appearance of autoantibodies specific to the CNS neurofilaments in patients with Alzheimer's and other CNS disorders suggests that the body's immune system may play a role in the disease process. S. Bahmanyar, R.K.H. Liem, J.W. Griffin and D.C. Gajdusek, *J. Neuropathol. Exp. Neurol.*, 53, p. 85-90 (1984); S. Bahmanyar, M.C. Moreau-Dubois, P. Brown, F. Catala and D.C. Gajdusek, *J. Neuroimmunol.*, 5, p. 191-196 (1983); T.S. Elizan, J. Casals and M.D. Yahr, *J. Neurol. Sci.*, 59, p. 341-347 (1983). The autoantibodies against normal CNS neurofilaments react with the paired helical filaments in neurofibrillary tangles characteristic of Alzheimer's Disease. D. Dahl and A. Bignami, *Exp. Neurol.*, 58, p. 74-80 (1978); M.E. Bruce, *J. Neuropathol. Exp. Neurol.*, 37, p. 595, abstract (1978).

Animal models for these CNS disorders, which are induced with aluminum chloride or B,B'-iminodipropionitrite (IDPN) to form paired helical filaments in neurofibrillary tangles, also react with antibodies directed against CNS neurofilaments. J.W. Griffen, P.N. Hoffman, A.W. Clark, P.T. Carroll and D.L. Price, *Science*, 202, p. 633-665 (1978).

Control of such autoimmune reactions may lead to the alleviation of symptoms manifested by such reactions. Over the past two decades, a body of clinical literature has accumulated relating to the treatment of autoimmune disease (or, more appropriately, diseases reflecting immune dysfunction) using a technique called provocative-neutralization therapy. Miller, *Annals of Allergy*, 38, p. 185-191 (1977); Miller, *Trans. Am. Soc. Opth. & Otolar. Allergy*, 14, p. 159-168 (1974); Miller, *Clinical Medicine*, 81, p. 16-19 (1974). In short, this methed, which is commonly employed for allergy therapy, involves subcutaneous or sublingual introduction of an antigen known, or suspected, to provoke symptoms reflective of immune dysregulation. By serial titration of the provoking material, a concentration of that agent may be determined which will neutralize those symptoms induced by the same substance at a different concentration. That is a prime example of a dose-dependent phenomenon in which one dose induces a positive reaction while another dose of the same agent induces a negative response.

Although it is thought that neutralization occurs as a consequence of reestablishing homeostatic functional levels of T8 suppressor cells, it is quite possible that the same antigen used at a neutralizing concentration to reverse immune dysregulation could also, or instead, trigger endocrine and/or neuronal control mechanisms to reverse symptoms. Because of the intimate association between the three control systems (endocrine, immune, nervous) and proven communication pathways between and among the cells comprising these respective systems, a single active molecule, such as amyloid beta protein in the Alzheimer's victim, and related CNS disorders, may reverse symptoms via any or all of these routes.

Plaque formation is a common component in the etiology of numerous other disease as well. Principal among those are arteriosclerotic diseases. Like Alzheimer's and related diseases, arteriosclerotic diseases,

such as atherosclerosis, are plaquing diseases. Such diseases are characterized by arterial plaque formation. These plaques commonly occur in large and medium-sized arteries and generally comprise cells, connective tissue (usually elastin, collagen, and glycosaminoglycans), and lipid deposits. The mixture of those components is usually complex, forming lesions which may be calcified in advanced stages of the disease. Plaque mass slowly increases throughout life, as blood vessels undergo progressive concentric fibromuscular thickening. In atherosclerotic patients, fibromuscular thickening of the intima of blood vessel walls proceeds rapidly and contributes, along with lipid deposition, to restricted blood flow. In non-atherosclerotic patients, the normal thickening of the walls of blood vessels does not contribute to increases in blood pressure and does not compromise blood flow. In fact, plaquing diseases often occur together and patients with neural plaques also have vascular plaques.

It is upon the matrix of fibromuscular thickenings that atherosclerotic plaques develop. Such plaques generally become more prevalent in the third decade of life, with localization being most common in the coronary arteries. Atherosclerotic lesions are generally thought to develop from fatty deposits which transiently occur in all humans in the developed muscular lining of blood vessels. The mechanism of transformation from fatty deposits or "streaks" to atherosclerotic lesions appears to be unknown. However, at least one report suggests that a virus may cause transformation of the normal lipid streaks to atherosclerotic plaques. Melnick, *et al.*, *JAMA*, *263*: 2204-207 (1990); wherein it was reported that an avian herpesvirus stimulated atherosclerotic lesions in chickens. The above-cited authors also correlated the presence of cytomegalovirus in humans with atherosclerotic lesions in humans. A finding of herpesvirus and cytomegalovirus antigens, as well as nucleic acids encoding those viruses, in arterial smooth muscle suggests that viral infection of arterial cells may be coincident with the development of

atherosclerosis. However, a causative relationship between any virus and atherosclerosis has yet to be conclusively determined.

Also of interest to the present invention is hypertension. The increases in vascular permeability generally observed in hypertension may increase influx of lipoprotein into cells, thus increasing the likelihood of atheroma formation. Hypertension may also contribute to atherosclerosis in blood vessels surrounding the brain. A reduction in hypertension has been shown to significantly reduce the incidence of myocardial infarction associated with atherosclerosis. Other factors in the development and progression of atherosclerosis include diabetes mellitus, which may reduce lipid efflux from cells in the arterial wall. In addition, cigarette smoking dramatically increases the risk of developing atherosclerosis and associated hypertension, including their sequelae, such as infarction of the myocardium and brain. Obesity is another factor which may contribute, especially in an individual who smokes. Overall, hypertension is the single greatest risk factor in coronary diseases as well as cerebrovascular stroke.

The presence of hypertension is a primary indicator of an arteriosclerotic condition and is often used by physicians as the sole diagnostic measure of diseases such as atherosclerosis. Moreover, a reduction of blood pressure is thought to have an effect in reducing the severity of atheroma plaques. The mechanism for such reduction may be a reduction in the transport of lipids and proteins into blood vessels which is coincident with a reduction in blood pressure. The diastolic component of blood pressure is generally thought to be the primary indicator of hypertension. While the systolic component may vary greatly depending upon nervousness, anxiety and the like, diastolic blood pressure generally remains constant and is more reflective of a patient's general vascular state. A diastolic reading of over 90 is considered mild hypertension in an adult and a diastolic reading of over 100 is considered hypertensive and an indicator of arteriosclerotic disease.

SUMMARY OF THE INVENTION

The present invention provides methods for alleviating the symptoms of disease states associated with plaque formation. In accordance with the invention, there is provided a method to stimulate the appropriate metabolic regulatory systems (immune, CNS or endocrine) which retard the progress of the symptoms of plaquing diseases, such as Alzheimer's and related diseases and arteriosclerotic diseases. Observations by scientists have now indicated that the apparent elevated amyloid beta protein concentration in, for example, Alzheimer's diseases may not be due to an increase in genomic expression, but possibly to activation of a mechanism that induces the reorganization of amyloid moieties from normal neurofilaments into paired helical filaments resulting in neurofibrillary tangles, neuritic plaques or amyloid plaques. D. Goldjaber, M.I. Lerman, O.W. McBridge, U. Suffiotti and D.C. Gaidusak, *Science*, 235, p. 77-780 (1987); R.E. Tanzi, J.F. Gusella, P.C. Watkins, G.A.P. Bruns, P. St.George, M.L. Vankeuren, D. Patterson, S. Pagan, D.M. Kurnit and R.L. Neve, *Science*, 235, p. 880-884 (1987); M. Baudry, B.R. Dubrin, L. Beasley, M. Leon and G. Lynch, *Neurobiol. Aging*, 7, p. 255-260 (1986); G.G. Glenner, *Arch. Path. Lab. Med.*, 107, p. 218-282 (1983). The mechanisms of the present invention may result in triggering control processes that correct the rearrangement of neurofilaments, alter abnormal amyloid protein formation including amyloid beta formation, and/or allow for clearing of axonal transport mechanisms. Similarly, regulatory control systems, as note above, play a role in arteriosclerotic plaque formation, leading to arteriosclerotic diseases such as atherosclerosis. A significant common occurrence in patients having arteriosclerotic disease and/or neural plaquing disease, such as Alzheimer's, is hypertension. Accordingly, methods of the present invention cause a reduction in hypertension as an indication of alleviation of the overall disease state. The diseases susceptible to treatment with

methods according to the invention have in common plaque formation. Accordingly, treatment with methods according to the invention provides an effective treatment of all such diseases by alleviating causative symptoms of the disease. In addition, as detailed below, compositions and methods of the invention are useful in the reduction of hypertension generally.

In a preferred embodiment of the invention, a composition is provided comprising amyloid protein and an influenza virus vaccine. In order to identify a dose for use in the invention, a wheal produced upon intradermal injection of the therapeutic material was evaluated according to criteria set forth in Moore, *Clinical Medicine*, *81*: 16-19 (1974), incorporated by reference herein. Upon subcutaneous injection, a wheal may be determined to be positive ten minutes after injection as a blanched, hard, raised, and discoid protrusion from the skin. A negative wheal is sufficiently absorbed at the end of ten minutes that the protrusion on the skin has grown less than an average of two millimeters in diameter from its original size.

In a preferred embodiment of the invention, compositions according to the invention comprise a dose from about 10^{-10} to about 10^{-2} mg of amyloid protein and about 0.05 cc of between a 1:5 and 1:125 dilution of an influenza virus vaccine in saline. Thus, the total volume of a typical composition according to the invention for administration to a patient is about 0.05 cc, or one drop. An influenza virus vaccine according to the invention may be any such vaccine, including a commercially-available vaccine, such as Fluogen™ (Parke Davis, Morris Plains, NJ) vaccine. Compositions according to the invention may comprise β-amyloid protein or the first 28 amino acids of β-amyloid protein.

Also in a preferred embodiment, compositions according to the invention are pharmaceutical compositions for treatment of arteriosclerotic diseases which pharmaceutical compositions comprise

amyloid protein and an influenza virus vaccine in a pharmaceutically-acceptable carrier.

Methods according to the present invention are useful in alleviating symptoms of arteriosclerosis generally, and atherosclerosis in particular. Such methods comprise the step of administering to a patient suspected or confirmed as having an arteriosclerotic disease an effective amount of a pharmaceutical composition comprising amyloid protein and an influenza virus vaccine. An effective amount of a composition according to the invention is an amount which results in a reduction in the symptoms of an arteriosclerotic disease. Most preferably, an effective amount of a composition according to the invention comprises from about 10^{-10} to about 10^{-2} mg of an amyloid protein, preferably β-amyloid protein, and about 0.05 cc of between a 1:5 and 1:125 dilution of an influenza virus vaccine. An amyloid protein used in methods according to the invention may be a β-amyloid protein or may be the first 28 amino acids of a β-amyloid protein. A highly preferred amount of amyloid protein used in compositions and methods according to the invention is from about 10^{-5} and about 10^{-2} mg of amyloid protein.

Methods and compositions according to the present invention are effective in alleviating symptoms of any disease in which plaquing is involved and especially diseases in which atheroma formation is characteristic. Compositions and methods according to the invention also alleviate hypertension and reduce cholesterol, both of which have a direct effect in reducing the severity of or eliminating symptoms associated with arteriosclerotic disease. The following detailed description of the invention provides exemplification of claimed methods and compositions. However, it is understood by the skilled artisan that other uses of the invention, specifically relating to the treatment of arteriosclerotic diseases, are within the scope of the present claims.

Also in a preferred embodiment, the invention provides a method for alleviating the symptoms of disease states associated with abnormal accumulation of and/or molecular organization of amyloid protein or amyloid plaques, which comprises administration to a diseased patient of an effective amount of amyloid protein or an effective active fragment thereof. The amyloid protein is preferably an amyloid beta protein although amyloid protein fragments such as fragments comprising the first 28 amino acid residues of the amyloid beta protein are expected to be useful. The method of the invention is useful against disease states associated with abnormal accumulation of and/or molecular organization of amyloid protein or amyloid plaques including disease states in which the amyloid protein or plaques are associated with the central nervous system and histopathologically related disorders. Such diseases include, but are not limited to Alzheimer's Disease and Parkinson's Disease. Other disease states include those such as atherosclerosis.

DETAILED DESCRIPTION OF THE INVENTION

A. Application of Materials and Methods of the Invention to the Treatment of Alzheimer's and Related Diseases

The alleviation of Alzheimer's Disease symptoms observed following administration of amyloid beta protein as described herein likely reflects stimulation of appropriate metabolic regulatory systems in the Alzheimer's Disease patients such that accumulation and/or formation of the paired helical filaments in neurofibrillary tangle and/or amyloid plaque developments are significantly altered or slowed and accumulated proteins are eliminated. This reprogramming to establish proper homeostatis would allow more efficient transmission of nerve impulses which would result in clinical improvement of treated Alzheimer's patients.

Typically, a pharmaceutical dosage unit of the present invention for the delivery of amyloid beta protein in a low concentration

comprises a liquid or solid carrier and an effective amount of amyloid beta protein. One suitable carrier for sublingual administration comprises a phenylated saline solution. Effective amounts of the amyloid protein range from about 10^{-10} to about 10^{-2} mg, and preferably from about 10^{-5} to about 10^{-3} mg and most preferably about 10^{-4} mg, amyloid beta protein in association with pharmaceutically acceptable excipients. The amyloid beta protein is administered through standard methods, including sublingual, subcutaneous and transdermal routes, and in dosage units that are either liquid or solid.

One explanation for the mode of action of this invention may be that the amount of this protein administered is sufficient to trigger a negative feedback mechanism to the body such that production of additional amyloid beta protein, possibly through breakdown of normal neurofilaments, is inhibited. Under this theory, the low level of amyloid beta protein, or a derivative thereof, gives a signal to the body to correct the abnormal synthesis/degradation process. The body sensors are then adjusted to normal metabolic control of amyloid beta protein processing that allows the proper balance to reestablish itself, alleviating the abnormal processing. The immune system, as well as the endocrine and CNS control systems, could play an integral regulatory role in response to the low dose therapy, with the amyloid protein functioning through mechanisms that not only correct the molecular organization of the amyloid beta protein moieties, but clear the interfering amyloid molecular constructs.

In a preferred embodiment, the present invention provides administration of amyloid beta protein or a derivative thereof. The amyloid beta protein may be provided either as part of a liquid solution or in a solid powder matrix, and may be administered with conventional excipients to permit ease of administration and accurate dosage delivery. Patients characterized herein below were evaluated using a battery of objective tests designed to measure cognitive ability. These included the mini-mental

State Examination, the Verbal Fluency Task Examination (word name task and category task), evaluation on the Dematis Dementia Rating scale, and the Word-Association Task Examination of the Wechster Memory Scale-Revised. Not all results of all tests are provided herein, however, the results of all the tests were qualitatively the same as those below and led to the same conclusions as those provided herein relative to the effect of treatment according to the invention.

EXAMPLE 1

A 67 year old male with a history of Alzheimer's Disease for four years prior to initiating therapy presented with an inability to answer questions, to place names with faces, and to complete his sentences. His wife noted a consistent downhill progression of his condition on a monthly basis. At the initiation of therapy, with the composition of the invention, his initial score on the Mini-Mental State Exam was 5 of a possible 30. The subject was treated by sublingual administration four times per day of a dosage unit comprising 10^{-4} mg of amyloid beta protein in a phenylated saline solution. After five months of therapy according to the invention, the patient scored a 12 1/2 on the Mini-Mental State Exam, was reading road signs while travelling and was communicating with family members. Also, the patient appeared to be more relaxed and better able to respond to his wife's efforts to assist him.

EXAMPLE 2

An 81 year old male with a history of Alzheimer's Disease was treated according to the invention. Prior to treatment the subject was unable to dress himself, had a flat affect, was poorly communicative and scored 10 1/2 on the Mini-Mental State Exam. The subject was treated by sublingual administration four times daily of the amyloid beta dosage unit of Example 1. After three months of treatment, the subject scored 17

points on the Mini-Mental State Exam, was more animated in speech, could dress himself most days and was more confident in physical actions.

EXAMPLE 3

In this example, the subject was a 62 year old female who was originally diagnosed by the University of Pittsburgh Medical School Alzheimer's Disease Research Center to be suffering from a fulminating form of Alzheimer's Disease. In one year, the subject had gone from being director of nursing in a chronic care establishment to requiring constant care. The subject was unable to communicate, did not appear to recognize anyone and had a score of 1.5 on the Mini-Mental State Exam. The subject was treated by sublingual administration four times daily of the amyloid beta dosage unit of Example 1. After three months of treatment, the patient's husband reported that warmth had returned to the patient's hands, no deterioration of any type was evident, although prior to therapy, he could note weekly declines. Communication remained difficult but improved for the subject, she showed increased alertness, and she was not only able to recognize individuals consistently, but also was able, at times, to participate in conversations, and her test score rose to 7.75 on the Mini-Mental State Exam. Although the subject was originally diagnosed as having Alzheimer's Disease, the results of an autopsy indicated that she did not have Alzheimer's Disease but suffered from a form of Parkinson's Disease known as Striatonigral degeneration.

EXAMPLE 4

In this example, the subject was a 79 year old male who had suffered from two transient ischemic attacks and had also been diagnosed as having Alzheimer's Disease. Prior to treatment, the subject had a score of 16 on the Mini-Mental State Exam. The subject was treated by sublingual administration four times daily of the amyloid beta dosage unit

of Example 1. While the subject became somewhat more irascible and eventually died of a stroke two months after the trial period, his performance on various mental performance exams including the Dementia Rating Scale improved during the initial six month evaluation period. Specifically, the Mini-Mental State Exam improved during the initial six months of testing with scores of 18, 20, 21 and 25 at the three, four, five and six month tests, respectively. The subject's mental performance at the three month follow-up examination had declined significantly, with the score on the Mini-Mental State Exam dropping to the level of the original test with a score of 16. The subject's scores on the other mental performance exams also showed marked decline to the original performance levels. The subject died of a stroke prior to the six-month follow-up evaluation.

EXAMPLE 5

In this example, a 76 year old male who was diagnosed as having Alzheimer's Disease, was treated by sublingual administration four times daily of the amyloid beta dosage unit of Example 1. The subject's performance on both the Mini-Mental State Exam and the Dementia Rating Scale improved significantly over five months of testing. Test scores on the Mini-Mental State Exam on the first, third, fourth and fifth months were: 20, 20, 20 and 25; while scores on the Dementia Rating Scale were: 115, 117, 122 and 126. The subject showed improvement primarily in areas of attention and conceptualization and in immediate short term memory, with some improvement in verbal fluency. In contrast, his performance did not improve in the area of delayed memory which remained severely compromised. In addition, there was only slight improvement demonstrated on tasks requiring new learning abilities which was also severely compromised. The subject was unavailable for follow-up study.

EXAMPLE 6

In this example, the subject was a 74 year old woman who was diagnosed as having Alzheimer's Disease so advanced that she was unable to identify a comb or a key. The subject was disoriented, incontinent and severely hypertensive and required intensive around the clock care from her sister who was a nurse. The subject was treated by sublingual administration four times daily of the amyloid beta dosage unit of Example 1. The subject's initial score on the Mini-Mental State Exam was 2, and the subject demonstrated slight improvements (subsequent scores were 2, 3, 4, 8 and 7) although some, but not all, of the improvement may have resulted from modification of the test procedure to accommodate the marked expressive language deficits exhibited by the subject. Significantly, the subject's blood pressure returned to normal upon treatment with the amyloid protein composition. This indicates that the amyloid protein composition exhibits utility in treatment of the symptoms of atherosclerosis which can be associated with amyloid plaques.

B. Application of Materials and Methods to the Treatment of Arteriosclerotic Diseases

Alzheimer's patients treated with amyloid protein as described above show a significant decrease in blood pressure as a result of such treatment which is concomitant with a reduction in symptoms of dementia. As noted above, Melnick, *et al.* report a role for a herpesvirus (Cytomegalovirus, CMV) in atherogenesis. In co-owned, United States Patent No. 4,880,626, it is noted that, while all untreated AIDS patients studied had CMV, none of the patients treated with a fluogen-based composition had CMV. The present application teaches compositions and methods comprising the anti-plaquing amyloid protein and the anti-viral influenza virus vaccine in order to effect treatment of patients presenting with arteriosclerotic conditions. The following examples provide

exemplification of the invention through representative embodiments comprising the use of claimed compositions and methods on human subjects. For each example below, original (*i.e.*, pretreatment) blood pressure was taken about three times during each reading to ensure accuracy. Subsequent measures were repeated about 5 times. Unless otherwise noted, patients undergoing treatment according to the invention received 1 drop (sublingual) four times per day. One drop is approximately 0.05 cc of a composition according to the invention.

EXAMPLE 7

A 54-year-old male patient presented with atherosclerosis, including blood pressure of 140/90. The patient was treated with sublingual drops of a composition comprising 10^{-9} mg amyloid protein in a 1:25 dilution of 0.05 cc fluogen™ in saline. The patient was not treated with any other medication during the period in which he was treated with the composition according to the invention. In addition, the patient reported that he remained on a high fat diet and reported no exercise during the treatment period. After daily sublingual treatment (1 drop 4 times per day) for 90 days, the patient's blood pressure had decreased to 117/72. After two years of taking the above composition, the patient's blood pressure has stabilized at about 115/70. The patient's cholesterol also significantly decreased after sustained treatment.

EXAMPLE 8

A 44-year-old, moderately obese male presented with blood pressure of 140/110 in early August, 1994. The patient was treated with daily sublingual doses (4 times daily) of a composition according to the invention, as recited above in Example 1. The patient received no other medication and did not otherwise alter his lifestyle during the treatment period. By early November, 1993, his blood pressure had decreased to

120/90. In April of 1992, after continued treatment as described above and with no change in lifestyle or diet during the treatment period, the patient had a blood pressure of 123/78. Blood pressure was taken up to five times during each measurement to ensure accuracy.

EXAMPLE 9

A 55-year old female with initial (pretreatment) blood pressure of about 138/90 began treatment according to methods of the invention and with compositions according to the invention in November, 1992. The patient showed a steady improvement in the diastolic component of blood pressure through three months of treatment. At that point, the patient discontinued treatment and three months later showed increases in diastolic blood pressure. Upon resuming treatment, diastolic blood pressure again decreased. During treatment, the patient was administered compositions as described above in Examples 1 and 2. The following table provides a partial tracking of the patient's blood pressure during treatment and non-treatment periods.

TABLE 1

Treatment	Date	Blood Pressure
pre-treatment	11/92	138/90
yes	1/93	150/88
yes	2/93	144/82
no	?	136/82
no	?	118/74
no	?	122/80
no	5/93	142/86
yes	1/94	140/80
yes	4/94	130/78

EXAMPLE 10

A 50-year-old male patient presented with blood pressure of 150/104 and began treatment as described above in June, 1992. The results of that treatment are presented in Table 2.

TABLE 2

Treatment	Date	Blood Pressure
yes	06/18/93	146/95
yes	06/25/93	155/94
yes	07/02/93	138/90
yes	08/06/93	138/78
yes*	09/93	150/98
yes*	09/93	140/96
yes*	09/93	156/108
no	10/27/93	160/102
no	11/04/93	160/100
yes	04/94	130/78

*dose of 1-2 drops/day

The results presented in Examples 3 and 4 demonstrate not only the effect of compositions according to the invention in reducing symptoms of arteriosclerosis, but also demonstrate that such symptoms return upon cessation of treatment according to the invention.

EXAMPLE 11

A 76-year-old female with blood pressure of 210/110 began treatment according to the invention and as described above, using 4 drops per day. After one month of treatment, the patient's blood pressure was reduced to 200/90. After 90 days of treatment her blood pressure was reduced to 160/90.

The foregoing representative results demonstrate that application of compositions according to the invention reduce blood pressure and other symptoms associated with arteriosclerosis. Therefore, treatment methods and compositions according to the invention constitute an effective means for alleviating the symptoms of arteriosclerotic diseases and for completely alleviating such diseases in some cases.

WHAT IS CLAIMED IS:

1. A method for alleviating the symptoms of disease states associated with amyloid plaque formation and/or formation of arterial plaques comprising administration to a patient of an effective amount of amyloid protein or a therapeutically active fragment thereof.

2. The method of claim 1 wherein the amyloid protein is an amyloid beta protein.

3. The method of claim 1 wherein the amyloid protein is a fragment comprising the first 28 amino acid residues of the amyloid beta protein.

4. The method of claim 1 wherein from about 10^{-10} to about 10^{-2} mg of amyloid protein is administered per dose.

5. The method of claim 1 wherein from about 10^{-5} to about 10^{-3} mg of amyloid protein is administered per dose.

6. The method of claim 1 wherein the disease state is associated with abnormal accumulation of and/or molecular organization of amyloid protein or amyloid plaques associated with central nervous system and histopathologically related disorders.

7. The method of claim 1 wherein the disease state is Alzheimer's Disease.

8. The method of claim 1 wherein the disease state is Parkinson's Disease.

9. The method of claim 1 wherein said effective amount of amyloid protein or a therapeutically-active fragment thereof is administered to a patient in composition also comprising an influenza virus vaccine.

10. The method of claim 9, wherein the disease state is atherosclerosis.

11. The method of claim 9, wherein said composition comprises from about 10^{-10} to about 10^{2} mg of amyloid protein and from about 0.05 cc of influenza virus vaccine at a dilution of between about 1:5 and about 1:125.

12. The method of claim 11; wherein said composition is administered to a patient in an amount of about 0.05 cc of the composition.

13. A pharmaceutical composition for treatment of disease states associated with abnormal accumulation of and/or molecular organization of amyloid protein or amyloid plaques which comprises amyloid protein or a therapeutically active fragment thereof in an amount effective to alleviate one or more symptoms of said disease state and a suitable carrier.

14. The pharmaceutical composition of claim 13 wherein the amyloid protein is amyloid beta protein.

15. The pharmaceutical composition of claim 13 wherein the amyloid protein is a fragment comprising the first 28 amino acid residues of the amyloid beta protein.

16. The pharmaceutical composition of claim 13 in the form of a single dosage unit which comprises from about 10^{-10} to about 10^{-2} mg of amyloid beta protein in association with pharmaceutically acceptable excipients.

17. The pharmaceutical composition of claim 13 further comprising an effective amount of influenza virus vaccine.

18. The pharmaceutical composition of claim 16; wherein said composition comprises from about 10^{-10} to about 10^{-2} mg of amyloid protein and from about 0.05 cc of influenza virus vaccine at a dilution of between about 1:5 and about 1:125.

19. A method for treating hypertension, comprising administration of an effective amount of the pharmaceutical composition of claim 18.

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 95/06689

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K39/145

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO,A,91 16819 (MOLECULARRX., INC.) 14 November 1991 see the whole document	1,2,4-7, 13,14,16 3,15
Y	SCIENCE, vol. 243, 17 March 1989 LANCASTER, PA US, pages 1488-1490, J.S. WHITSON ET AL. 'AMYLOID BETA PROTEIN ENHANCES THE SURVIVAL OF HIPPOCAMPAL NEURONS IN VITRO.' see the whole document	3,15

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 1995	29.09.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Ryckebosch, A

Form PCT/ISA/210 (second sheet) (July 1992)

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US95/06689

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-12,19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-12 and 19 are ditected to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Int. anal Application No
PCT/US 95/06689

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9116819	14-11-91	EP-A-	0526511	10-02-93
		JP-T-	6502387	17-03-94

Form PCT/ISA/210 (patent family annex) (July 1992)